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DETERMINATION OF CHLOROPHYLL CONTENT OF SEA GRAPES *(CAULERPA RACEMOSA)* IN NATURAL DEEP EUTECTIC SOLVENT (NADES) EXTRACT FROM GLUCOSE AND GLYCEROL

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Abstract

The aim of this research was to determine the chlorophyll content in the extract of *Caulerpa racemosa* using Natural Deep Eutectic Solvent (NADES) made from glucose and glycerol in various molar ratios. *C. racemosa,* a green alga known for its high chlorophyll content, was harvested from Jepara, Central Java, Indonesia. The algae samples were divided into four parts: rhizoid, stolon, ramuli, and a mixture of the three. Extraction was conducted using NADES at glucose molar ratios of 1:1, 1:2, and 1:3, with 40% water added. NADES was prepared by heating at 65°C with a stirring speed of 200 rpm until a homogeneous liquid formed. The chlorophyll a, chlorophyll b, and total chlorophyll content in the extract were analyzed using a UV-Vis spectrophotometer at wavelengths of 649 nm and 667 nm. It was found that NADES with a glucose molar ratio of 1:2 was the most effective, yielding the highest chlorophyll content in the ramuli part at 13.571 mg/L. Meanwhile, extraction with NADES glucose (1:3) in the rhizoid part resulted in the lowest chlorophyll content at 0.566 mg/L. Significant differences in chlorophyll content based on the molar ratio of NADES and the thallus part extracted were indicated by statistical analysis using ANOVA. The potential of NADES as an environmentally friendly and effective solvent for chlorophyll extraction from C. racemosa was confirmed by this research. New opportunities for the application of NADES in the development of sustainable pharmaceutical and nutraceutical products, with non-toxic and environmentally friendly extracts, were opened by these findings.

Keywords: *C. Racemosa*, Chlorophyll Extraction, Green Solvents, NADES, Sustainable Extraction.

INTRODUCTION

C. racemosa, one of the green algae, is known for its rich chlorophyll content, making it attractive due to its potential applications in various industries, including food, medicine, and cosmetics (1). The extraction of chlorophyll from *C. racemosa* is an interesting topic, and the use of Natural Deep Eutectic Solvents (NADES) has emerged as a promising method for efficient extraction (2). Composed of natural compounds such as glucose and glycerol, NADES have demonstrated high solubility for biomolecules, making them a sustainable and environmentally friendly alternative to conventional organic solvents (3).

Chlorophyll is a crucial pigment for photosynthesis, known for its distinct absorption spectrum, with various types such as chlorophyll f and d exhibiting unique characteristics when exposed to specific light wavelengths (4). Chloroplasts, where most chlorophyll is located, play a significant role in the biosynthesis of various metabolites and phytochemicals, underscoring the importance of understanding the dynamic metabolic processes within plant cells (5). Additionally, the extraction of chlorophyll from *C. racemosa* using NADES offers opportunities to explore the interactions between the solvent and chlorophyll compounds, potentially leading to

improved extraction efficiency (2,6). Furthermore, the use of NADES in the extraction of bioactive compounds, including chlorophyll, has become a promising research area. Published study focused on the valorization of olive mill waste through the recovery of polyphenols with NADES, demonstrating the potential of these solvents in sustainable waste management (7). Additionally, published article proposed the application of non-canonical redox cofactors in fermentation processes, indicating ongoing exploration of novel approaches in extraction and metabolism processes (8). These references collectively highlight the growing interest in utilizing NADES for sustainable extraction processes and their potential to address environmental and waste management challenges.

The utilization of NADES for chlorophyll extraction aligns with the increasing interest in environmentally friendly techniques for extracting bioactive compounds, emphasizing the need for sustainable and efficient extraction methods (9). Moreover, the potential antimicrobial phototoxicity of chlorophyll dissolved in NADES adds another dimension to the potential applications of the extracted chlorophyll. Understanding the extraction behavior of chlorophyll in NADES and its potential antimicrobial properties is essential for exploring the diverse applications of chlorophyll extracts. Therefore, this article aims to determine the chlorophyll content of *C. racemosa* in NADES extracts made from glucose and glycerol with different compositional ratios.

MATERIALS AND METHODS

Collection of samples

In this study, fresh samples of *C. racemosa* were used. The *C. racemosa* utilized was cultivated macroalgae obtained from Jepara, Central Java, Indonesia. The *C. racemosa* was harvested at 45 days of age. All parts of the *C. racemosa* thallus, consisting of rhizoids, stolons, and ramuli, were used as samples. The samples of *C. racemosa* are shown in Figure 1.

Figure 1: Sea grapes (*C. racemosa*) used in research

Preparation of the Sample

The fresh *C. racemosa* samples were separated into four parts: rhizoids, stolons, ramuli, and a mix (rhizoids, stolons, ramuli) using scissors. All samples were cleaned with water to remove dirt, moss, sand, and soil. The cleaned samples were then dried using tissue paper and ground to a fine powder using a mortar. The samples were

then ready to be extracted using NADES in a shaker incubator and subsequently analyzed for chlorophyll content using a UV-Vis spectrophotometer.

Natural Deep Eutectic Solvent (NADES) Preparation

Natural Deep Eutectic Solvent (NADES) was prepared following the method by (10) with modifications and preliminary tests, as listed in Table 1. NADES was made from a mixture of glucose and glycerol with the addition of water (40%). The molar ratios of glucose to glycerol were prepared at 1:1, 1:2, 1:3, 2:1, and 3:1 using an analytical balance. The mixture was heated to 65°C with a constant stirring speed of 200 rpm using a shaker incubator until a clear homogeneous liquid was formed. The NADES was allowed to cool to room temperature before being stored in storage bottles for further analysis. The pH value of NADES was determined using a pH meter (Eutech CyberScan pH 300). The viscosity of NADES was measured under controlled stress conditions and with a shear rate of 1 s^{-1} using a Myr VR 3000 Viscometer, with measurements taken at room temperature and at 40°C. The density of NADES was also measured using an Ohaus Pioneer Analytical instrument at room temperature and at 40°C.

Extraction of Chlorophyll from *C. racemosa*

The extraction of chlorophyll from fresh *C. racemosa* was conducted in the laboratory. The extraction method was based on published methods with modifications (11–13,13,14). An incubator shaker was used for the extraction. A 5 g sample was weighed, then ground with a mortar until smooth, and 50 ml of NADES was added. The sample-to-NADES ratio was 1:10. The mixture was then placed in an incubator shaker and extracted for 30 minutes at 40°C with a speed of 200 rpm. After extraction, the mixture was filtered using filter paper, and the obtained filtrate was centrifuged at 15,000 rpm for 15 minutes at 4°C. The supernatant was then collected and its absorbance at wavelengths of 649 nm and 667 nm was measured using a UV-Vis spectrophotometer (15). All extraction activities were carried out in a dark room.

Analysis of Chlorophyll Content Using UV–Vis Spectrophotometer

The chlorophyll extract solution in NADES from *C. racemosa* was analyzed for chlorophyll a, chlorophyll b, and total chlorophyll content. The chlorophyll content in the NADES extract was measured using a UV-Vis spectrophotometer (Jasco V-760, measurement wavelength range of 187–900 nm, bandwidth of 1 nm, spectral resolution of 0.1 nm, and stray light lower than 0.00008%) at absorbances of 649 nm and 667 nm (16). The chlorophyll content was calculated based on the following formulas:

$$Ca = 13.95 \times D_{667} - 6.88 \times D_{649}$$
$$Cb = 24.96 \times D_{649} - 7.32 \times D_{667}$$
$$Ct = Ca + Cb$$

Where D_{649} and D_{667} are the absorbance values at 649 nm and 667 nm, respectively. Ca is the chlorophyll a content (mg/L), Cb is the chlorophyll b content (mg/L), and Ct is the total chlorophyll content (mg/L).

Statistical Interpretation

The data analysis employed two-way ANOVA using SPSS 26 to assess the significance of chlorophyll content in NADES-extracted *C. racemosa* from different thallus parts (ramuli, stolons, rhizoids, and mix) and its relationship with the use of NADES with varying compositions of glucose and glycerol ratios (1:1, 1:2, 1:3, 2:1, 3:1).

RESULTS AND DISCUSSION

The differences in molar ratios between glucose and glycerol with the addition of 40% water were used to create 5 NADES samples, and their outcomes are described in Table 1. NADES were prepared by mixing all ingredients and stirring at 200 rpm below 65°C for 120 minutes.

Table 1: Composition and Abbreviations of NADES

Molar ratio (glucose : glycerol)	Water (%)	Abbreviation	Appearance
1 : 1	40	Nades 1	Colorless liquid
1 : 2	40	Nades 2	Colorless liquid
1 : 3	40	Nades 3	Colorless liquid
2 : 1	40	Nades 4	White precipitate
3 : 2	40	Nades 5	White precipitate

From the 5 NADES samples prepared, 3 successful NADES were obtained: Nades 1, Nades 2, and Nades 3, characterized by the formation of colorless liquid after being left at room temperature for 24 hours. Meanwhile, the compositions of the other 2 NADES, Nades 4 and Nades 5, were unsuccessful, indicated by the presence of white precipitates after being left at room temperature for 24 hours. Nades 4 and Nades 5 were unsuccessful because the two components could not mix well, resulting in the formation of white precipitates. The high concentration of hydrogen bond donors in glucose compared to glycerol made the mixture heterogeneous and formed a semi-solid phase. Upon cooling at room temperature, it formed a solid white phase (17). This leads to the conclusion that Nades 4 and Nades 5 are not recommended and were not used in this study. Based on this, the study also focused on measuring the physical properties of NADES (viscosity, density, and pH).

The formation of natural deep eutectic solvents (NADES) involves a delicate balance of components to achieve successful mixtures. In a study by (18), it was observed that out of 5 NADES samples prepared, 3 were successful, namely Nades 1, Nades 2, and Nades 3, which resulted in the formation of colorless liquid after 24 hours at room temperature. On the other hand, Nades 4 and Nades 5 were unsuccessful due to poor mixing of components, leading to the formation of white precipitates. This failure was attributed to the high concentration of hydrogen bond donors in glucose compared to glycerol, causing the mixture to be heterogeneous and form a semi-solid phase, eventually solidifying into a white phase upon cooling.

As a result, Nades 4 and Nades 5 were deemed unsuitable for the study (18). The study not only focused on the successful NADES but also delved into measuring the physical properties of these solvents, including viscosity, density, and pH. This emphasis on characterizing the physical properties of NADES aligns with the broader interest in understanding the diverse applications and behaviors of these green solvents. The research by sheds light on the importance of proper formulation and

compatibility of components in NADES to achieve desired properties and avoid undesired outcomes (18). In the realm of green chemistry, the use of NADES has gained attention as sustainable and eco-friendly solvents.

These solvents, composed of natural metabolites, offer a promising alternative to traditional organic solvents. The published article by (18,19) highlights the potential of NADES in various applications, emphasizing their role as green solvents with unique properties derived from natural sources. The chemical diversity of natural metabolites contributing to NADES formation underscores the significance of these solvents in biological and chemical processes (19,20). In conclusion, the successful formulation of NADES requires a careful selection of components to ensure compatibility and desired properties. Understanding the behavior and characteristics of NADES, as demonstrated by (18), is crucial for their effective utilization in various fields. The research by (18,19) further underscores the importance of NADES as sustainable solvents with diverse applications, paving the way for greener and more environmentally friendly chemical processes.

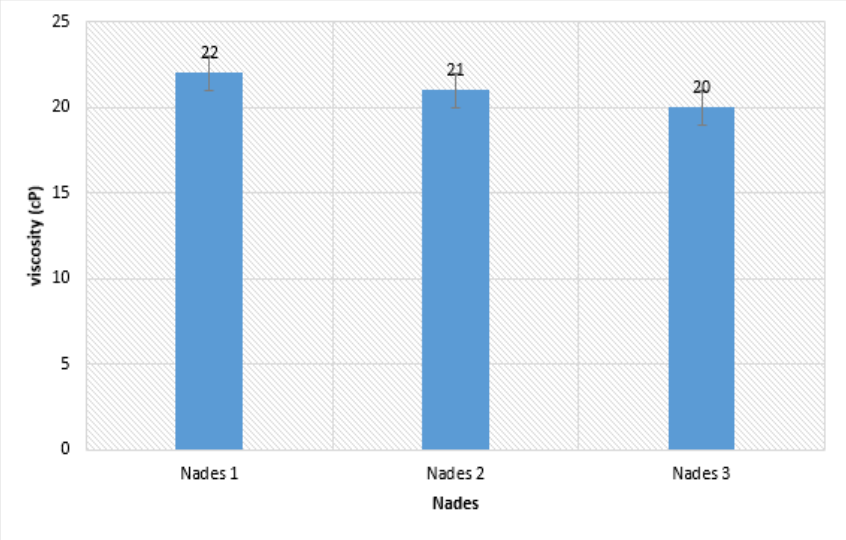

Figure 2: NADES viscosity at room temperature

In Figure 2, it is shown that the viscosity decreases from Nades 1 to Nades 3. The viscosity values of Nades 1, Nades 2, and Nades 3 are 22 cP, 21 cP, and 20 cP, respectively. This indicates that the addition of glycerol molar ratio to these NADES weakens the hydrogen bond interactions between glucose and glycerol, resulting in a decrease in viscosity. Viscosity in Natural Deep Eutectic Solvents (NADES) is greatly influenced by the presence of hydrogen bond interactions between hydrogen bond donors (HBD) and acceptors (HBA) in the solvent mixture. The formation of strong hydrogen bonds between these components contributes to the high viscosity of NADES, impacting the solvent's solubility and extraction efficiency. The ability of NADES to form hydrogen bonds enhances its effectiveness in extracting bioactive compounds from natural materials, as viscosity affects the mass transfer kinetics during the extraction process. Understanding the relationship between viscosity and hydrogen bonding in NADES is crucial for optimizing their use in environmentally friendly extraction methodologies (21). High NADES viscosity is often associated with extensive hydrogen bond interactions between components. The decrease in NADES viscosity is a result of weakening hydrogen bond interactions between components

(18). This aligns with the observation that as the glycerol molar ratio increases in NADES from Nades 1 to Nades 3, the viscosity decreases from 22 cP to 20 cP, indicating a weakening of hydrogen bond interactions between glucose and glycerol (22). The ability of NADES to form hydrogen bonds enhances its effectiveness in extracting bioactive compounds from natural materials, as viscosity plays a crucial role in mass transfer kinetics during the extraction process. This is supported by the work of (23), who emphasize that deep eutectic solvents (DES), including NADES, are recognized as green solvents due to their low cost, non-toxicity, and high biodegradability, making them suitable for various industrial applications (23).

In the context of optimizing the extraction of bioactive compounds using NADES, (24) discuss the use of various solvents, including Natural Deep Eutectic Solvents (NADES), for bioactive extraction from tobacco waste powder. This highlights the versatility and potential of NADES in extracting valuable compounds from different sources, showcasing their applicability in sustainable extraction processes (25). Furthermore, the study by (26) on the green extraction of phenolics and terpenoids from passion fruit peels using NADES underscores the innovative nature of these solvents, which are produced by heating a mixture of natural hydrogen bond acceptors and donors with a proper molar ratio. This emphasizes the environmentally friendly aspect of NADES and their potential in extracting bioactive compounds from natural sources (24).

In conclusion, the references provide a comprehensive understanding of the relationship between viscosity, hydrogen bonding, and the effectiveness of NADES in extracting bioactive compounds. The decrease in viscosity observed with increasing glycerol molar ratio in NADES aligns with the weakening of hydrogen bond interactions between components, highlighting the importance of these interactions in the properties and applications of NADES in green extraction methodologies.

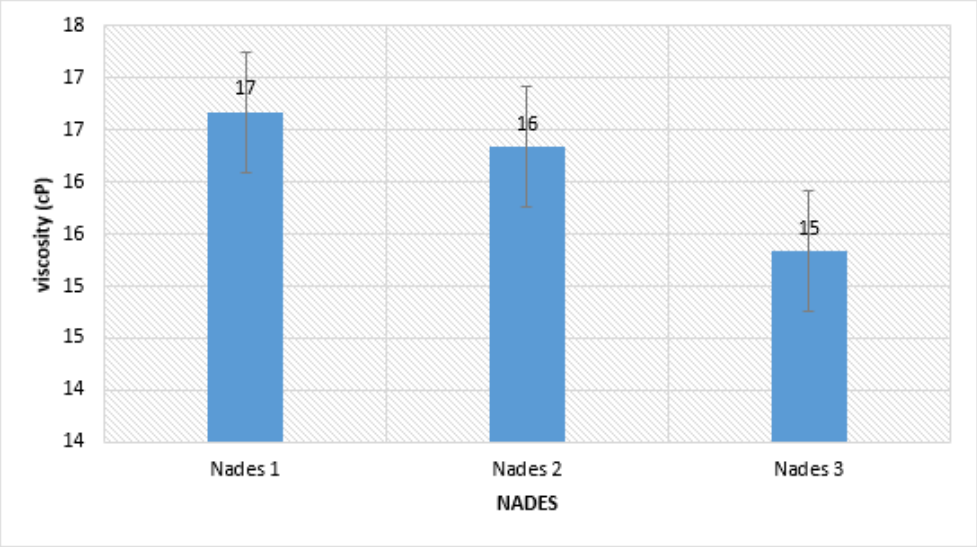

Figure 3: NADES viscosity at 40°C

Heating can affect the viscosity of NADES. In Figure 3, it is shown that there is a decrease in NADES viscosity when heated to 40°C. Nades 1, Nades 2, and Nades 3 all experience a decrease in viscosity when compared to their viscosities at room temperature. The viscosities of Nades 1, Nades 2, and Nades 3 when heated to 40°C are 17 cP, 16 cP, and 15 cP, respectively. According to (27), an increase in

temperature can decrease NADES viscosity, facilitating solvent penetration, enhancing the release of target compounds for easy extraction, and increasing extraction capacity. This change in viscosity can occur because an increase in temperature will enhance diffusion and the total solute's solubility, disrupt matrix bonds in the solute, and reduce the viscosity and surface tension of the solvent mixture, thereby enhancing penetration into the solid matrix and increasing surface area. Thus, the influence of heating on NADES viscosity can play an important role in the extraction process of bioactive compounds.

The influence of temperature on the viscosity of Natural Deep Eutectic Solvents (NADES) and its implications for the extraction process of bioactive compounds can be supported by the work of (28,29). (28) observed that NADES viscosity decreases with increasing temperature and water content, aligning with the idea that an increase in temperature can lead to a reduction in viscosity, facilitating solvent penetration and enhancing the release of target compounds for extraction. Similarly, (29) highlighted that as the temperature increases, the intermolecular forces in NADES intensify, leading to a reduction in viscosity, which can enhance the extraction efficiency of bioactive compounds. Furthermore, the study by (30) is relevant as it mentions that even small amounts of water can significantly decrease the viscosity of most NADES, indicating that changes in water composition can impact the viscosity of these solvents. This aligns with the concept that altering the water content in NADES can influence their physical properties, such as viscosity, which in turn affects the extraction efficiency by improving mass transfer rates. In conclusion, the references provide insights into how temperature affects the viscosity of NADES, emphasizing that an increase in temperature can lead to a decrease in viscosity, thereby facilitating solvent penetration, enhancing the release of target compounds for extraction, and increasing extraction capacity. Understanding the relationship between temperature, viscosity, and extraction efficiency is crucial for optimizing the extraction process of bioactive compounds using NADES.

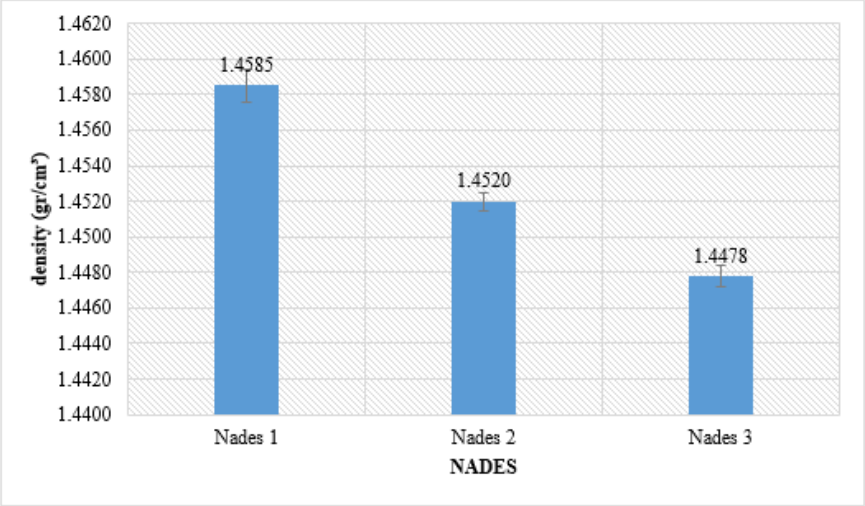

Figure 4: NADES density at room temperature

Figure 4 illustrates that the density at room temperature decreases with the addition of glycerol molar ratio. A range of density values is obtained, ranging from 1.4480 g/cm³ to 1.4520 g/cm³. The densities of Nades 1, Nades 2, and Nades 3 are 1.4585 g/cm³, 1.4520 g/cm³, and 1.4480 g/cm³, respectively. NADES density can be

influenced by the number of hydroxyl groups and the length of the carbon chain. NADES density will increase with the increase in hydroxyl groups and the length of the carbon chain on the HBD. The presence of aromatic groups on the HBD causes NADES density to decrease (19,30)

To further elaborate on the relationship between the density of Natural Deep Eutectic Solvents (NADES) and the influence of their composition, particularly the addition of glycerol molar ratio, it is essential to consider the impact of various components on the density of NADES. (19,31,32) discuss that the density of NADES can be affected by factors such as the number of hydroxyl groups and the length of the carbon chain in the hydrogen bond donor (HBD) component. They note that an increase in hydroxyl groups and carbon chain length on the HBD leads to an increase in NADES density, while the presence of aromatic groups on the HBD can cause a decrease in density. This aligns with the observation that the density of NADES decreases with the addition of glycerol molar ratio. Moreover, NADES are characterized by tailorable physicochemical properties that can be adjusted by changing the type and molar ratio of their constituents (33). This flexibility allows for the customization of NADES properties, including density, by modifying their composition. The ability to tune the density of NADES through compositional adjustments is crucial for various applications, including extraction processes where density can influence solute solubility and mass transfer rates. In summary, the density of NADES is intricately linked to the composition of these solvents, with factors such as the number of hydroxyl groups, carbon chain length, and aromatic groups playing significant roles in determining the density. Understanding how changes in composition impact the density of NADES is essential for optimizing their properties for specific applications, such as the extraction of bioactive compounds.

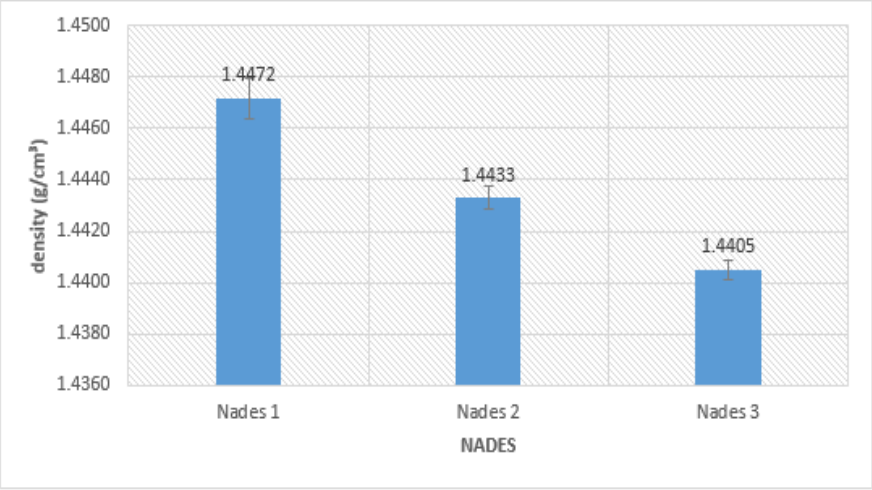

Figure 5: NADES Density at 40°C

Figure 5 shows a decrease in NADES density when heated to 40°C compared to the density of NADES at room temperature. With the increase in glycerol molar ratio, the density decreases. From the results of this study, the density of NADES at 40°C ranges from 1.4405 g/cm³ to 1.4472 g/cm³. Density is influenced by the molar ratio of components with specific ratios leading to lower NADES density and melting points (17). Glucose-based NADES density changes with temperature changes, and the relationship between density and temperature is nonlinear. Thus, understanding how temperature affects density is crucial for utilizing NADES in various industrial

applications. To delve deeper into the impact of temperature and glycerol molar ratio on the density of Natural Deep Eutectic Solvents (NADES), it is essential to consider the research findings of (17,34). The density of NADES is affected by the molar ratio of components, with specific ratios leading to lower density and melting points (17,22). This is consistent with the observation that the density of NADES decreases with an increase in glycerol molar ratio and when heated to 40°C compared to room temperature. Additionally, published article mention that NADES solvents, such as those derived from choline chloride and sorbitol, exhibit lower melting points due to their eutectic nature, enabling them to remain in a liquid state at room temperature (34). Moreover, the study by (35) is pertinent as it underscores the significance of physical-chemical parameters, including density, in the extraction process when utilizing NADES. The ability to manipulate the density of NADES through compositional adjustments is critical for enhancing extraction efficiency by influencing factors such as solute solubility and mass transfer rates. In conclusion, the density of NADES is intricately tied to the composition of these solvents, with temperature and glycerol molar ratio playing pivotal roles in determining their density. Understanding how alterations in temperature and composition affect the density of NADES is imperative for their effective application in various industrial processes, particularly in extraction procedures. The influence of Natural Deep Eutectic Solvents (NADES) selection and thallus part selection on the chlorophyll content of fresh *C. racemosa* seaweed, along with the statistical analysis of chlorophyll extraction results, can be supported by the research of (36). (36,37)discuss the nutritional value and biofunctionalities of edible green seaweeds, including *C. racemosa*, emphasizing the presence of UVB-absorbing compounds in *C. racemosa*. This study provides insights into the potential photoprotective mechanisms in tropical green seaweeds, which can be linked to the chlorophyll content variations observed in different parts of *C. racemosa*.

Additionally, the work of (38) is relevant as it analyzes the nutritional and health values of *C. racemosa*, shedding light on the minerals, pigments, and antioxidants present in this seaweed species. Understanding the biochemical composition of *C. racemosa* is crucial for elucidating its chlorophyll extraction properties and the impact of NADES selection on chlorophyll yield. In conclusion, the references by (36–38) provide valuable insights into the nutritional composition and biofunctionalities of *C. racemosa*, which can help explain the variations in chlorophyll extraction results based on NADES selection and thallus part selection.

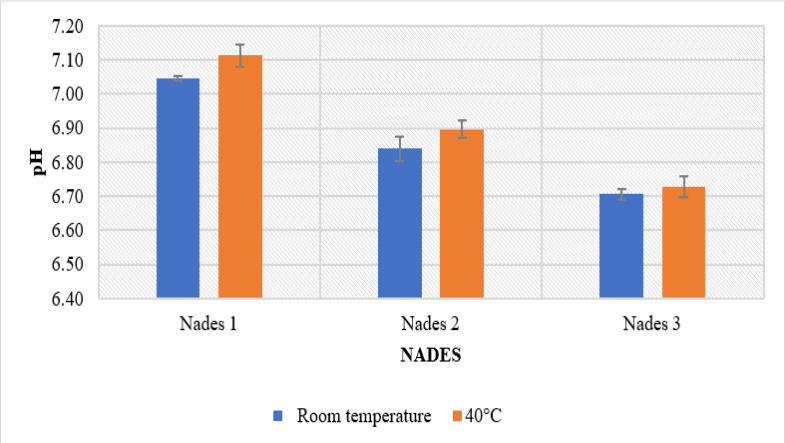

Figure 6: NADES pH at room temperature and 40°C

In Figure 6, the pH of NADES measured at room temperature is in the range of 6.71 to 7.05. Meanwhile, the pH of NADES measured at 40°C shows values ranging from 6.73 to 7.11. From the results of this study, it can be concluded that the pH of NADES with a glucose and glycerol ratio composition falls within the neutral pH range. This is consistent with the findings of (39), which state that NADES containing sugar or polyalcohol have pH values close to neutral. The pH of NADES plays an important role in influencing its physicochemical properties. The pH of NADES can affect extraction efficiency and selectivity for various compounds. Additionally, the pH of NADES can influence the stability and bioactivity of extracted compounds, making it suitable for various applications in pharmaceutical and nutraceutical product development (40). The pH of NADES is a crucial factor that influences solubility, extraction efficiency, selectivity, stability of extracted compounds, as well as their toxicity and environmental impact. After understanding the physicochemical properties of NADES, they were used to extract chlorophyll from *C. racemosa*. Three NADES formed from glucose and glycerol were each used to extract each part of the *C. racemosa* thallus. The thallus was divided into 4 parts: rhizoids, ramuli, stolons, and mix (rhizoids, ramuli, stolons). The calculation of the total chlorophyll content of the extraction results is presented in Table 2.

Table 2: Total chlorophyll extracted

Interaction of NADES and thallus part	Total Chlorophyll (mg/L)	Std. Error
A1B1	3.820	0.032
A1B2	1.404	0.032
A1B3	6.542	0.032
A1B4	6.171	0.032
A2B1	5.007	0.032
A2B2	13.571	0.032
A2B3	8.407	0.032
A2B4	7.509	0.032
A3B1	0.566	0.032
A3B2	2.151	0.032
A3B3	0.789	0.032
A3B4	1.476	0.032

Note: Values with different letters (a, b, c, etc.) are considered significantly different ($p < 0.05$).

A1 = Nades 1 (glucose/glycerol, 1:1), A2 = Nades 2 (glucose/glycerol, 1:2), A3 = Nades 3 (glucose/glycerol, 1:3). B1 = rhizoid, B2 = ramuli/assimilator, B3 = stolon, B4 = mix (rhizoid, ramuli, stolon).

The results indicate that there is an influence of NADES selection for extraction and thallus part selection on the chlorophyll content of fresh *C. racemosa* seaweed. Testing and statistical analysis of the extraction results of *C. racemosa* chlorophyll show that the use of NADES and thallus parts show differences ($p < 0.05$) in extracting chlorophyll in *C. racemosa* samples. Table 1 shows that the highest chlorophyll extraction result from Caulerpa racemose using NADES is 13.571 mg/L obtained from the extraction of ramuli/assimilator parts using glucose/glycerol (1 : 2) NADES. This indicates that glucose/glycerol (1 : 2) is the appropriate molar ratio where glucose as a hydrogen donor can bind perfectly with glycerol, thus able to dissolve chlorophyll compounds (19). The ability of NADES to form hydrogen bonds with plant cell wall components such as cellulose and hemicellulose allows it to disrupt cell wall structure and release trapped bioactive compounds within the cell (18). This interaction helps

to break down barriers posed by plant cell walls, thus enabling the extraction of various compounds, including chlorophyll, from plant tissues. It is also supported that ramuli/assimilator parts are upright branches from stolons, having many small spherical structures resembling grapes. These branches play a role in photosynthesis and nutrient absorption, hence this part potentially contains the most chlorophyll (41). The smallest chlorophyll extraction result was obtained from treatment A3B1, namely NADES with glucose/glycerol (1 : 3) composition on the Rhizoid part. This result is significantly different from other total chlorophyll results indicated by different letter notations. This is possibly because glucose/glycerol (1 : 3) NADES may not be able to penetrate the cell walls of Caulerpa racemose, thus unable to extract chlorophyll effectively.

CONCLUSION

In this study, glucose NADES was investigated as an alternative solvent that is more environmentally friendly for the extraction of high-value products from natural raw materials. This research successfully combined the characterization of glucose with the addition of 40% water for its application in chlorophyll extraction from *C. racemosa* seaweed. In this regard, the extraction showed promising results in terms of obtaining chlorophyll extract in the supernatant with its chlorophyll content calculated. To our knowledge, this is the first-time chlorophyll extraction from *C. racemosa* has been conducted using NADES solvents. The advantages obtained from this extract are non-toxic, food-grade, and environmentally friendly results.

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